



The Crustacean Society

Journal of
Crustacean Biology*Journal of Crustacean Biology* (2018) 1–14. doi:10.1093/jcobiol/ruy057

Molecular phylogenetic analysis of the devil crayfish group, with elevation of *Lacunicambarus* Hobbs, 1969 to generic rank and a redescription of the devil crayfish, *Lacunicambarus diogenes* (Girard, 1852) comb. nov. (Decapoda: Astacoidea: Cambaridae)

Mael G. Glon^{1,✉}, Roger F. Thoma^{2,✉}, Christopher A. Taylor³,
Marymegan Daly^{1,✉} and John V. Freudenstein^{1,✉}

¹The Ohio State University, Museum of Biological Diversity, 1315 Kinnear Road, Columbus, OH 43212, USA;

²Midwest Biodiversity Institute, 4673 Northwest Parkway, Hilliard, OH 43026, USA; and

³Illinois Natural History Survey, 1816 S. Oak Street, Champaign, IL 61820, USA

Correspondence: M. Glon; email: glon.1@osu.edu

(Received 10 April 2018; accepted 7 June 2018)

ABSTRACT

As North American crayfish biodiversity becomes increasingly imperiled, the ambiguous nature of the current taxonomic framework can impede conservation efforts. The taxonomy of the family Cambaridae Hobbs, 1942 has historically been based on morphology, but recent studies using molecular phylogenetic techniques have revealed taxonomic inconsistencies, including a polyphyletic *Cambarus* Erichson, 1846. We took a step towards increasing the taxonomic resolution of Cambaridae by investigating a group of primary burrowing crayfishes which were historically part of the *Cambarus* subgenera *Lacunicambarus* Hobbs, 1969 and *Tubericambarus* Jezerinac, 1993. This group, here called the devil crayfish group (DCG) because it includes the devil crayfish (*Cambarus diogenes* Girard, 1852), has a confusing taxonomic history and is in need of revision to inform conservation assessments. We tested the hypothesis that the DCG is monophyletic through phylogenetic analyses of mitochondrial DNA sequence data from multiple specimens of the eight DCG species and a broad sample of taxa representing approximately 70% of the species in what is currently recognized as *Cambarus*. Seven of the eight species from the DCG form a well-supported, monophyletic group that is distinct from the remainder of what has traditionally been recognized as *Cambarus*. Our analyses place the DCG as sister to a clade consisting of taxa from *Creaserinus* Hobbs, 1973, *Faxonius* Ortmann, 1905, and *Barbicambarus* Hobbs, 1969. Based on our results and on unique morphological and ecological characteristics, we split seven of the eight DCG species from *Cambarus* and elevate the subgenus *Lacunicambarus* to generic level to accommodate them. We redescribe *Lacunicambarus* and the devil crayfish *sensu stricto* (*Lacunicambarus diogenes* comb. nov.) and designate a neotype for the species to facilitate subsequent revisionary work.

Key Words: Astacidea, Cambaridae, conservation, *Lacunicambarus*, North America, revision, systematics, taxonomy, *Tubericambarus*

INTRODUCTION

Crayfish biodiversity is becoming increasingly imperiled by threats that include habitat loss and biological invasions (Richman *et al.*, 2015; Bland, 2017). These threats are especially pronounced in North America, where approximately 32% of crayfish species are

ranked as endangered or vulnerable by the American Fisheries Society's Endangered Species Crayfish Subcommittee (Taylor *et al.*, 2007). The plight of North American crayfishes has not gone unnoticed, as evidenced by the recent addition of two species to the Federal List of Endangered and Threatened Wildlife

2.5 (Loughman *et al.*, 2015b; U.S. Fish and Wildlife Service, 2016). One of the major impediments to the conservation of North American crayfishes, however, is taxonomic uncertainty (Dubois, 2003; Loughman & Fetzer, 2015; Owen *et al.*, 2015; Loughman *et al.*, 2017b). The ambiguous nature of the current taxonomic framework makes it difficult to properly assess the conservation status of North American crayfishes as it exposes measures of range and population size to misestimation and maintains the possibility of undescribed or under-studied taxa being wholly overlooked (Soltis & Gitzendanner, 1999; Wheeler, 2004; Crandall *et al.*, 2008).

2.10 The taxonomy of North American crayfishes has historically been based on morphology, which does not always reflect the evolutionary history of species as a result of convergent evolution (Crandall & Fitzpatrick, 1996; Taylor & Knouft, 2006). Recent studies have used molecular analyses to investigate the taxonomy of North American crayfishes from the family Cambaridae and found multiple instances where generic classifications did not correspond to monophyletic groups (e.g., Taylor & Knouft, 2006; Breinholt *et al.*, 2012; Crandall & De Grave, 2017). While some of the genera within Cambaridae have been rearranged in light of information generated by molecular phylogenetic techniques (e.g., all surface-dwelling crayfishes that were previously in *Orconectes* Cope, 1872 have been reassigned to the resurrected *Faxonius* Ortmann, 1905; Crandall & De Grave, 2017), others remain unresolved. *Cambarus* Erichson, 1846 is one whose monophyly has recently come into question, but thus far the only corresponding taxonomic act has been the discontinuation of use of subgenera within this genus pending additional data (Johnson *et al.*, 2011; Breinholt *et al.*, 2012; Crandall & De Grave, 2017). While the reasoning behind the general rejection of *Cambarus* subgenera outlined in Crandall & De Grave (2017) is cogent, increased sampling may reveal reasons to formally recognize particular clades from within *Cambarus*.

2.20 We increase the taxonomic resolution of the genera within Cambaridae by investigating a group of burrowing crayfishes that has historically been part of *Cambarus*. This group, which we hereafter refer to as the devil crayfish group (DCG), is made up of eight species of primary burrowing crayfishes that belonged to the rejected subgenera *Lacunicambarus* Hobbs, 1969a (i.e., devil crayfish, *C. diogenes* Girard, 1852; painted devil crayfish, *C. ludovicianus* Faxon, 1884; rusty gravedigger, *C. milhus* Fitzpatrick, 1978; warpaint mudbug, *C. erythrodactylus* Simon & Morris, 2015) and *Tubericambarus* Jezerinac, 1993 (i.e., thornytail crayfish, *C. acanthura* Hobbs, 1981; little brown mudbug, *C. thomai* Jezerinac, 1993; paintedhand mudbug, *C. polychromatus* Thoma, Jezerinac & Simon, 2005; cocoa crayfish, *C. stockeri* Thoma 2011). In all previously published phylogenies that have included members of the DCG, representative specimens from *Tubericambarus* have been nested inside of *Lacunicambarus*, with the combined taxa from both subgenera forming a monophyletic clade basal to the rest of *Cambarus* (with one exception that we address below). No study to date has presented a phylogeny that includes all eight taxa of the DCG, so the monophyly of this group has not yet been explicitly tested.

2.25 We took particular interest in the DCG because of the turbulent taxonomic history of the devil crayfish, which is currently considered the most widely distributed crayfish in North America (found in 32 U.S. states and Ontario, Canada; Hobbs, 1969a; Taylor *et al.*, 2007) but has long been suspected of being a species complex (e.g., Faxon, 1885; Ortmann, 1906a; Hobbs, 1989; Jezerinac, 1993). Owing to the historical ambiguity surrounding the devil crayfish, it is difficult to know whether early accounts of this species refer to the devil crayfish *sensu stricto* or to one of the other species from the DCG that have only recently been described or remain undescribed. This issue is exacerbated by the absence of the holotype for the devil crayfish, as the type designated by Girard was destroyed in the Great Chicago Fire of 1871 (Faxon, 1885). The devil crayfish is currently listed as

“demonstrably widespread, abundant, and secure” by The Nature Conservancy and as “Currently Stable” by the American Fisheries Society Crayfish Subcommittee (Taylor *et al.*, 2007). Until a systematic revision is conducted to examine the diversity within the DCG, however, the accuracy of these conservation status assessments remains uncertain because individual species constituting this complex may have small ranges and require differential conservation attention (Bickford *et al.*, 2007).

2.80 We took the first steps towards a systematic revision of the DCG by testing the hypothesis that the DCG forms a monophyletic group via phylogenetic analyses using new molecular data generated from multiple specimens of all eight species in the DCG combined with molecular data generated by Breinholt *et al.* (2012). These data represent approximately 70% of the species in what is currently recognized as *Cambarus*. Based on our results, we split seven of the eight DCG species from *Cambarus* and place them in *Lacunicambarus*, which we herein elevate to generic rank and whose original description we amend to also encompass species that were previously in the subgenus *Tubericambarus*. We also redescribe the devil crayfish *sensu stricto* (*Lacunicambarus diogenes* (Girard, 1852) comb. nov.) and designate a neotype for the species to facilitate subsequent revisionary work.

MATERIALS & METHODS

Sampling

2.100 We focused our sampling on the eight species that were previously grouped into *Lacunicambarus* and *Tubericambarus*, placing special emphasis on collecting specimens of each species from its type locality and across its range. We located chimneys at the mouths of crayfish burrows in roadside ditches or stream banks and floodplains and then excavated them by hand or with a small gardening pickaxe. We also captured a subset of specimens by hand while wading in roadside ditches at night when they were out of their burrows. Immediately following capture, we slid forceps underneath the cephalothorax and into the branchial chamber of each crayfish to remove one or two gills, which we placed into individually labelled tubes of 100% ethanol. We transferred sample tubes to a laboratory freezer (−20 °C) as soon as possible to prevent DNA degradation and placed the remaining specimens into jars with 70% ethanol, which we later catalogued and deposited in the crustacean collection of Ohio State University Museum of Biological Diversity (OSUMC). We also obtained some samples from researchers at other institutions as well as from collections, including the Illinois Natural History Survey (INHS; samples from abdominal muscle rather than gill tissue) and the Smithsonian National Museum of Natural History (USNM; Supplementary material Table S1).

DNA extraction, PCR, and sequencing

2.125 We dried tissue samples for one hour at 22 °C, then used a plastic pestle to pulverize the samples in individual PCR tubes. We extracted total genomic DNA using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer’s recommended protocols. We used polymerase chain reaction (PCR) following the guidelines outlined in Crandall & Fitzpatrick (1996) to amplify three mitochondrial loci: partial 12S rDNA (approximately 400 bp using primers 12sf and 12sr; Mokady *et al.*, 1994), partial 16S rDNA (approximately 460 bp using the primers 16sf-cray and 1472-reverse; Buhay & Crandall, 2005, Crandall & Fitzpatrick, 1996), and partial CO1 (approximately 650 bp using primers LCO1-1490 and HCO1-2198; Folmer *et al.*, 1994). Following PCR, we used gel electrophoresis to examine the quality and size of our PCR products, and sent aliquots to TacGen (Richmond, CA, USA) for sequencing in both directions on an ABI 3730XL DNA analyzer using BigDye Terminator v3.1 sequencing chemistry.

3.5 *Sequence analyses*

We used Geneious R11 (www.geneious.com; Kears *et al.*, 2012) to correct and assemble our DNA sequences, all of which we uploaded to GenBank (see [Supplementary material Table S1](#) for accession numbers). We also downloaded from GenBank 251 additional sequences of 12S, 16S, and partial CO1 from 84 crayfish taxa (representing the genera *Astacus* [Fabricius, 1775](#), *Barbicambarus* [Hobbs, 1969](#), *Cambarellus* [Ortmann, 1905](#), *Cambarus*, *Creaserinus* [Hobbs, 1973](#), *Faxonella* [Creaser, 1933](#), *Faxonius*, *Pacifastacus* [Bott, 1950](#), and *Procambarus* [Ortmann, 1905](#); see [Breinholt *et al.* 2012: Appendix S1](#) for data on the specimens for which we downloaded sequences) that were generated by [Breinholt *et al.* \(2012\)](#) to make inferences about *Cambarus*. To ensure that our partial CO1 sequences did not contain potentially problematic nuclear mitochondrial pseudogenes (i.e., numts; [Richly & Leister, 2004](#)), we translated them to look for stop codons and mapped them to an annotated crayfish mitochondrial genome downloaded from Genbank. We performed individual MUSCLE ([Edgar, 2004](#)) alignments with a maximum number of eight iterations on each locus in Geneious.

3.15 *Phylogenetic analyses and hypothesis testing*

For each of our three loci, we used jModelTest 2 ([Darriba *et al.*, 2012](#); [Guindon & Gascuel, 2003](#)) with three substitution schemes to compute likelihood scores for 24 candidate nucleotide substitution models. We then performed model selection using the Akaike Information Criterion corrected for small sample size (AICc) and Bayesian Information Criterion (BIC). We inferred phylogenetic trees using maximum likelihood in IQ-Tree ([Nguyen *et al.*, 2015](#); [Chernomor *et al.*, 2016](#)) and Bayesian inference in MrBayes ([Huelsenbeck & Ronquist, 2001](#); [Ronquist & Huelsenbeck, 2003](#)). We prepared our data for IQ-Tree by dividing it into separate partitions by locus and designating a substitution model for each partition based on the results of jModelTest 2 (HKY+G for 12S and 16S, GTR+I+G for partial CO1). We used 1,000 bootstraps to establish nodal support in the tree and estimated model parameters as well as branch lengths independently for each partition. We prepared our data for MrBayes by dividing it into partitions and designating the same substitution models as for IQ-Tree. We unlinked the variables statefreq, revmat, shape, and pinvar for all partitions and enabled partitions to evolve under different rates. We ran a Markov-Chain Monte Carlo (MCMC) analysis using two independent runs with one cold chain and three heated chains for 5,000,000 generations with sampling every 500 generations. We checked for convergence by monitoring the standard deviation of the split frequencies and discarded the first 25% of samples as burn-in. We combined the two independent runs after discarding the burn-in and created a majority-rule consensus tree with nodal support for the trees assessed using posterior probabilities.

3.55 *Note on lack of nuclear DNA sequences*

It was originally planned to include a nuclear locus in our analyses to better resolve the deeper relationships in our phylogeny. To make any meaningful intergeneric inferences would have required nuclear DNA data not just from our focal DCG taxa but also from the other *Cambarus* taxa that we included in our phylogeny. Collecting specimens across such a wide range of *Cambarus* taxa to generate new nuclear data would have been prohibitively time- and cost-intensive. This left us with the option of using the 28S rDNA data generated by [Breinholt *et al.* \(2012\)](#) for the same specimens as our other sequences. [Breinholt *et al.* \(2012\)](#) found that 28S was only 0.3% divergent, however, and therefore contributed very little resolution relative to mitochondrial loci (i.e., 11.6% for 12S, 14.4% for 16S, and 18.7% for partial CO1; [Breinholt *et al.*, 2012](#)). To confirm that 28S would not have meaningfully influenced tree

topology or nodal support, we downloaded all specimens ($N = 52$) from the [Breinholt *et al.* \(2012\)](#) data set for which 12S, 16S, 28S, and partial CO1 data existed and used the same IQ-TREE maximum likelihood methods and MrBayes Bayesian inference mentioned above to make trees with and without 28S. We visually inspected these trees and did not find any meaningful differences to tree topology or nodal support, and therefore did not further pursue this locus.

3.85 RESULTS

Our maximum likelihood tree had a log-likelihood value of -23713.84 . The standard deviation of the split frequencies dropped below 0.01 during our Bayesian analysis, indicating convergence. Each of our two independent MrBayes runs generated 10,001 trees, 2,500 of which were discarded as burn-in, which left 15,002 trees in our post-burnin posterior distribution. The topologies of our Maximum Likelihood (ML) and Bayesian Inference (BI) trees have no significantly supported disagreements. We therefore present in all of our figures our ML tree annotated with both Bayesian posterior probabilities (PP_{BI}) and ML bootstrap values (B_{ML}).

Both analyses found maximal support for a monophyletic clade including all species from the DCG except for *C. stockeri* ($PP_{BI} = 1$, $B_{ML} = 99$; [Figs. 1, 2](#)). In both analyses, this clade is sister to a clade that includes the outgroup specimens from *Barbicambarus*, *Creaserinus*, and *Faxonius*, but with varying levels of support ($PP_{BI} = 1$, $B_{ML} = 46$; [Fig. 1](#)). *Cambarus* forms two maximally supported clades ($PP_{BI} = 1$, $B_{ML} = 100$; [Figs. 1, 3](#)) in both of our analyses. The first of these clades (hereafter *Cambarus* clade 1) is sister to the DCG and outgroup clades mentioned above ($PP_{BI} = 0.8$, $B_{ML} = 28$) and contains nearly all *Cambarus* specimens in our dataset (including the type species for the genus, *C. bartonii* ([Fabricius, 1798](#))). Of particular interest is the placement of all three *C. stockeri* specimens as sister to the three specimens from the rejected subgenus *Glareocola* [Bouchard & Bouchard, 1995](#) ($PP_{BI} = 0.94$, $B_{ML} = 44$; [Fig. 3](#)) and the placement of all three of our specimens of *C. deweesae* [Bouchard & Etnier, 1979](#) in a clade consisting of crayfishes from the eliminated subgenus *Depressicambarus* [Hobbs, 1969](#) (to which *C. deweesae* was originally assigned), and a single specimen from the rejected subgenus *Jugicambarus* [Hobbs, 1969a](#) (*Cambarus gentryi* [Hobbs, 1970](#)). The second *Cambarus* clade (*Cambarus* clade 2), which is sister to all taxa mentioned above, consists only of three troglobitic crayfishes from Arkansas, Missouri, and Oklahoma ($PP_{BI} = 1$, $B_{ML} = 54$; [Figs. 1, 3](#)).

DISCUSSION

This study is the first to explicitly examine the evolutionary relationship between the devil crayfish group (DCG) and *Cambarus* and presents the first phylogeny to include all currently-recognized species from the DCG complex. Seven of the eight species from the DCG form a well-supported, monophyletic clade that is distinct from the remainder of what has traditionally been recognized as *Cambarus*. Although we were not successful in resolving the backbone of our phylogeny with high confidence, both of our analyses place the DCG clade as sister to a clade consisting of taxa from *Creaserinus*, *Faxonius*, and *Barbicambarus*. This result supports previous studies that found that *Cambarus* is paraphyletic ([Johnson *et al.*, 2011](#); [Breinholt *et al.*, 2012](#); [Crandall & De Grave, 2017](#)), and confirms our hypothesis that the DCG is monophyletic and distinct from *Cambarus*.

The most complete phylogenetic representation of the DCG prior to our study was that of [Breinholt *et al.* \(2012\)](#), which presented a phylogeny containing approximately 70% of the species of *Cambarus*, including a single specimen from the subgenus *Tuberiacambarus* and nine from the subgenus *Lacunicambarus*. The

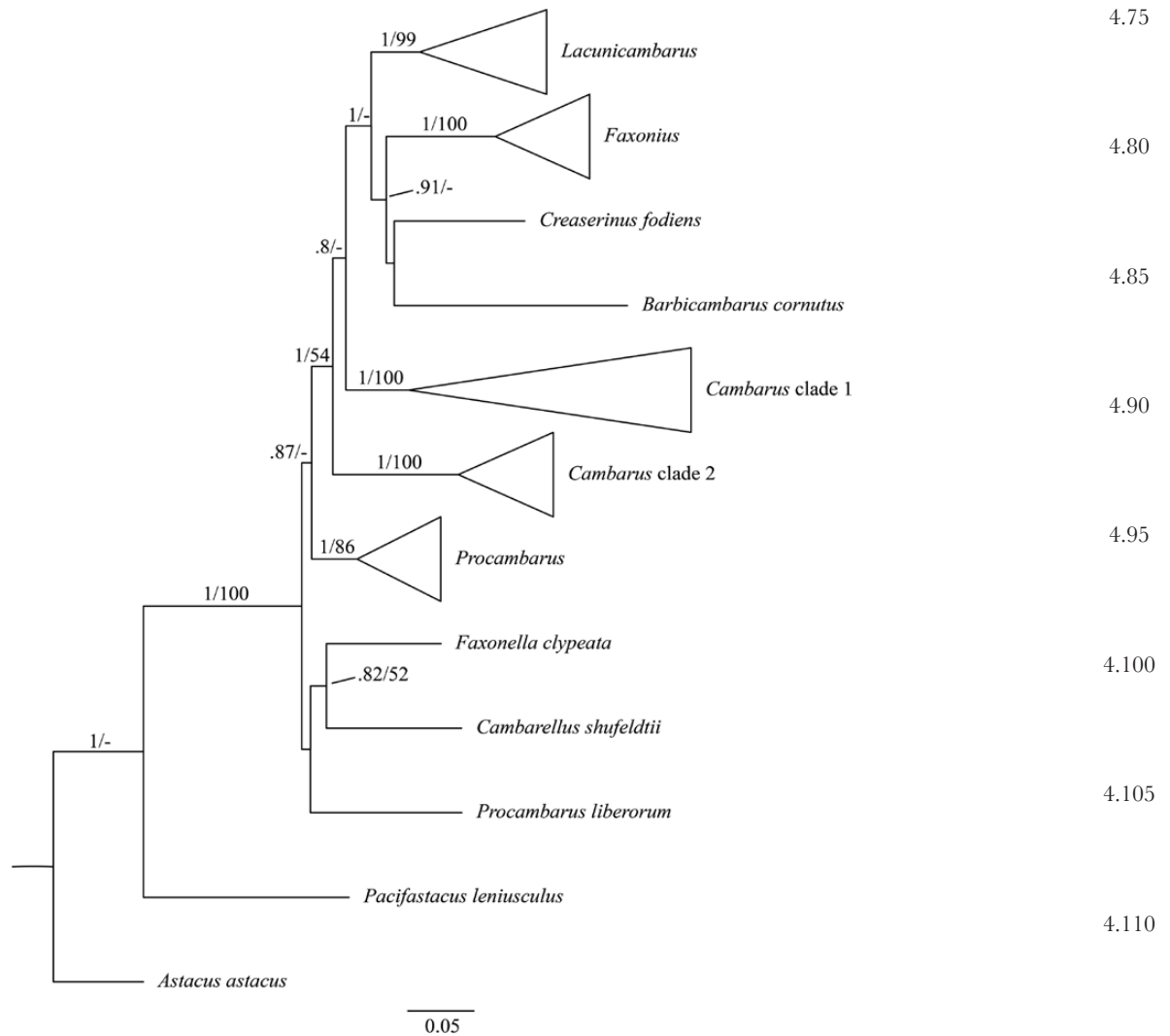


Figure 1. Phylogram showing maximum likelihood tree of complete dataset estimated from three mitochondrial loci (12S, 16S, and partial CO1). Support values > 0.5 Bayesian posterior probability and > 50 maximum likelihood bootstrap values are given in that order. Dashes indicate lack of support from one analysis and the absence of any annotation indicates lack of support from both analyses. Clades at the genus level are collapsed into triangles to improve legibility.

single specimen of *Tubicambarus* (*C. acanthura*) in the phylogeny of Breinholt *et al.* (2012) was sister to a specimen of *C. devesae*, and these two were nested within a clade that was otherwise only composed of the *Lacunicambarus* specimens. This placement rendered *Lacunicambarus* paraphyletic and was part of the basis for the rejection of this and the other *Cambarus* subgenera (Crandall & De Grave, 2017). The monophyly of *Tubicambarus* could not be similarly assessed due to the lack of other representative samples from this subgenus, but its phylogenetic validity was also questioned due to its placement within *Lacunicambarus*. The phylogenies of Breinholt *et al.* (2012), as does ours, nevertheless showed that the clade containing *Lacunicambarus*, *Tubicambarus*, and *C. devesae* was maximally supported and distinct from *Cambarus*.

One of the first steps towards establishing the monophyly of the DCG was therefore to investigate the placement of *C. devesae*, a primary burrowing crayfish from Tennessee and Kentucky (Bouchard & Etnier, 1979; Taylor & Schuster, 2004). We included in our analyses three specimens of *C. devesae* collected approximately 8 km from its type locality in Anderson County, Tennessee. In both analyses, our *C. devesae* specimens are nested within *Cambarus* clade 1. This group contains several members of the rejected subgenus *Depressicambarus*, the group to which *C. devesae*

was originally assigned (Bouchard & Etnier, 1979). In contrast, the *C. devesae* sequences from Breinholt *et al.* (2012) are placed in the well-supported clade that contains our sequences of *C. acanthura*. We were unable to procure the focal *C. devesae* specimen studied by Breinholt *et al.* (2012) to examine its anatomy or sequence additional genes. This specimen was only tentatively identified as a burrowing *Cambarus* spp. by a student in the field, however, and was therefore subject to possible misidentification (K. Crandall, personal communication). In light of the differences in placement of the sequences identified by Breinholt *et al.* (2012) as *C. devesae* and those we collected and considering that the range of *C. acanthura* extends into Anderson County, Tennessee (OSUMC 2425), we have high confidence that the sequences identified as being from *C. devesae* in Breinholt *et al.* (2012) came from a specimen of *C. acanthura*. We therefore do not consider *C. devesae* to be a member of the DCG and keep it in *Cambarus*.

A second step towards establishing the monophyly of the DCG was to further examine the phylogenetic validity of the rejected subgenera *Lacunicambarus* and *Tubicambarus*. *Lacunicambarus* was erected by Hobbs (1969a) in a pivotal revision of the genus *Cambarus*. At the time of the revision by Hobbs (1969a), the only members of the DCG that had been described were *C. diogenes*

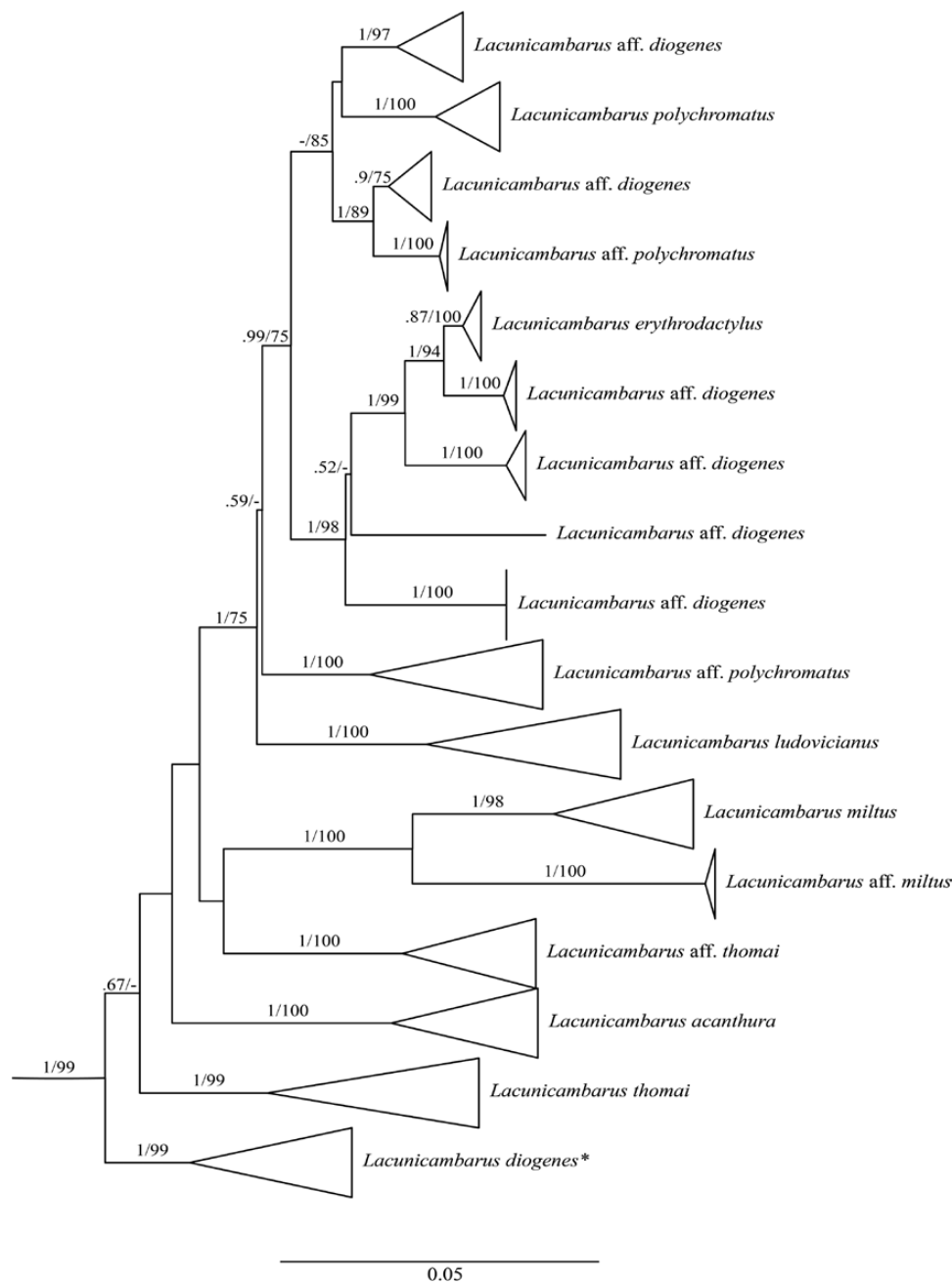


Figure 2. Phylogram showing maximum likelihood tree of *Lacunicambarus* estimated from three mitochondrial loci (12S, 16S, and partial CO1). Support values > 0.5 Bayesian posterior probability and > 50 maximum likelihood bootstrap values are given in that order. Dashes indicate lack of support from one analysis and the absence of any annotation indicates lack of support from both analyses. Clades at the species level are collapsed into triangles to improve legibility. The asterisk denotes the clade containing the type species of the genus, *Lacunicambarus diogenes*.

and *C. ludovicianus*; subsequently, *C. miltus* and *C. acanthura* were also placed in this subgenus. In his description of *C. thomai*, Jezerinac (1993) outlined morphological variation between specimens from species of *Lacunicambarus* and created the subgenus *Tubericambarus* to include *C. acanthura*, *C. thomai*, and an undescribed species referred to as *C. sp. A* (later described as *C. polychromatus*; Thoma et al., 2005). The primary distinguishing characteristic between these two subgenera was the tuberculation of the mesial and dorsomesial surfaces of the chelae: *Lacunicambarus* has two parallel rows of well-developed tubercles plus a third row running diagonally to a knob at the base of the dactyl, whereas *Tubericambarus* has dense tuberculation that covers the mesial 1/3 to 1/4 of the dorsomesial margin of the palm without forming distinct rows

(Jezerinac, 1993). Two new species were subsequently described and placed in *Tubericambarus* (*C. polychromatus* and *C. stockeri*; Thoma et al., 2005, Thoma, 2011) and one new species was placed in *Lacunicambarus* (*C. erythrodictylus*; Simon & Morris, 2015). Although we have frequently found the tuberculation of the mesial and dorsomesial surfaces of the chelae informative for species-level identification of members of the DCG, our analyses did not support this trait as diagnostic of monophyletic groups. The placement of *Tubericambarus* taxa within *Lacunicambarus* renders the latter paraphyletic, and the multiple *Tubericambarus* clades within *Lacunicambarus* render the former polyphyletic.

Our analyses revealed maximal support for a monophyletic DCG clade that includes all species other than *C. stockeri*, which

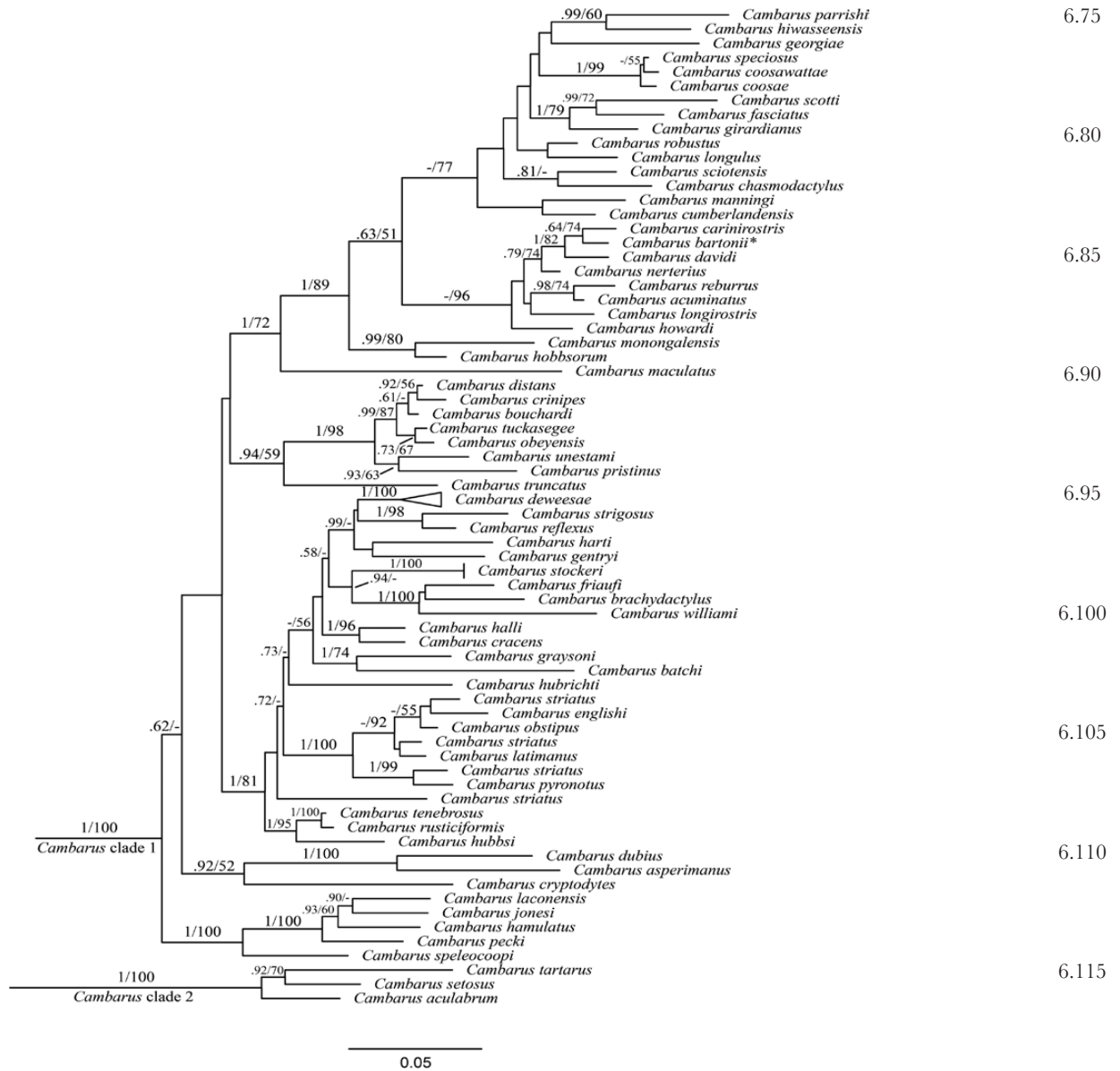


Figure 3. Phylogram showing maximum likelihood tree of the paraphyletic genus *Cambarus* estimated from three mitochondrial loci (12S, 16S, and partial CO1). Support values > 0.5 Bayesian posterior probability and > 50 maximum likelihood bootstrap values are given in that order. Dashes indicate lack of support from one analysis and the absence of any annotation indicates lack of support from both analyses. Clades at the species level are collapsed into triangles to improve legibility. The asterisk denotes the type species of the genus, *Cambarus bartonii*.

is placed in *Cambarus* clade 1. Thoma (2011), in his description of *C. stockeri*, expressed uncertainty about the subgeneric classification of this species due to plesiomorphic character states, including the heavy tuberculation of the entire chelae (a trait which is not found in any members of the DCG). Thoma (2011) placed this species in *Tubericambarus* but suggested that this was a tentative placement that should be further evaluated using genetic data. Although the rejection of the *Cambarus* subgenera renders the subgeneric placement of this crayfish moot, its relationship to members of the DCG is still of interest. We concur with Thoma (2011) that members of this species have a unique morphology that differentiates them from most other currently known species of *Cambarus*. Some of these morphological characteristics differentiate *C. stockeri* from the other members of the DCG, including the heavy tuberculation on the entire chela (rather than just part of the mesial or dorsomesial margin), the relatively long terminal elements of the gonopods of Form 1 males, and the narrowly open areola

(Thoma, 2011). These characters do not seem to correspond any better with the crayfishes from the rejected subgenus *Glareocola*, to which *C. stockeri* is sister to in our analyses. This peculiar species will require further examination, but based on the results of our analysis, we confidently exclude it from the DCG.

The DCG forms a maximally supported clade distinct from the remainder of what has traditionally been recognized as *Cambarus*. There are also a number of morphological and ecological characteristics that distinguish the DCG from *Cambarus* and further justify its recognition as a separate genus. Members of the DCG are distinguished by their prominent suborbital angles, obliterated or linear areola, rounded and robust chelae with either of two tuberculation patterns and a broad concavity in the proximal opposable margin of the dactyl, and Form 1 male gonopods with two short terminal elements recurved at about 90° relative to the shaft. While some members of the DCG are drab (e.g., *C. thomai*), others display vivid colors and patterns that are very distinctive (e.g.,

7.5 *C. ludovicianus* and *C. miltus*). Members of the DCG are widely distributed and typically associated with lowland stream and river floodplains and wetlands, where they preferentially burrow in clay soils, although habitat degradation has caused them to adapt to anthropogenic environments such as roadside ditches (Grow & Merchant, 1980; Hamr, 2006). The habitat and broad geographical range of the members of the DCG, along with occasional catches of DCG specimens in streams, suggests that these crayfishes are able to disperse via streams and rivers (Helms *et al.*, 2013; Miller *et al.*, 2014). This mode of dispersal contrasts with that of many other primary burrowing crayfishes, whose distribution is limited by large bodies of water (Ortmann 1906b; Loughman *et al.*, 2017a). None of these ecological or morphological characteristics by themselves are unique to the DCG, but together they characterize the group and serve to broadly distinguish it from other North American crayfishes.

7.20 As evidenced by previous studies and our results, *Cambarus* is polyphyletic and in need of taxonomic revision (Johnson *et al.*, 2011; Breinholt *et al.*, 2012; Crandall & De Grave, 2017). We carve the DCG out of this polyphyletic *Cambarus* and elevate the subgenus *Lacunicambarus* to generic rank. In doing so, we amend its original description to also encompass the DCG species that were previously in the subgenus *Tubericanbarus*. This ameliorates the taxonomic and phylogenetic mismatch for the DCG but does not completely remedy the problems of *Cambarus*. As a result of the placement of *C. bartonii*, the type species of *Cambarus*, in *Cambarus* clade 1, we suggest that subsequent studies evaluate the merit of recognizing *Cambarus* clade 2 as a separate genus from *Cambarus* clade 1. Doing so would possibly render *Cambarus* monophyletic; however, approximately 30% of the species included in this genus are missing from our phylogeny so we cannot definitively conclude that the genus will not need additional taxonomic attention as sampling is increased.

SYSTEMATICS

Family Cambaridae Hobbs, 1942

Genus *Lacunicambarus* Hobbs, 1969

7.45 The subgenera *Lacunicambarus* and *Tubericanbarus* are not phylogenetically independent as *Lacunicambarus* is paraphyletic with respect to the polyphyletic *Tubericanbarus*. Given that they cannot be distinguished, we consider them synonyms. Per Article 23.3 of the International Code of Zoological Nomenclature (ICZN, 1999), a genus formed by the union of two subgenera takes the oldest available name for either taxon, so we designate *Lacunicambarus* Hobbs, 1969, which precedes *Tubericanbarus* Jezerinac, 1993, as the name of this genus. Because the diagnosis of the subgenus *Lacunicambarus* given by Hobbs (1969a) is not pertinent to the genus *Lacunicambarus* as a whole, we provide an amended diagnosis for *Lacunicambarus*, which includes the seven currently described species in the genus.

7.60 *Diagnosis* (amended from Hobbs, 1969a and Jezerinac, 1993): Eyes pigmented, variable in size. Antennae not heavily fringed on mesial border. Rostral margins moderately thickened, floor excavated; lacking carina, marginal spines, marginal tubercles. Postorbital spines absent. Cervical spines usually absent, but faintly present or reduced to tubercles in some populations. Suborbital angle prominent, typically acute to subacute. Branchiostegal spine usually absent or reduced to small tubercle. Areola obliterated or linear among most of its length, constituting 37–49% of total length of cephalothorax, never bearing more than 2 punctations in narrowest part. Chela moderately robust with palm shorter than dactyl; dorsomesial surface of palm either with 2 parallel rows of 6–8 well-developed tubercles, plus third row running diagonally from mesial base of palm to lateral margin of dactyl articulation, additional scattered tubercles present between second and third row, or dorsomesial 1/4–1/3 studded with

tubercles not forming distinct rows; remaining dorsomesial surface tuberculate medially, punctate laterally; lateral margin of fixed finger of chela smooth, subcostate or costate with punctations, but never bearing row of spines; fingers typically gaping with well-defined dorsomedian longitudinal ridges; proximal opposable margin of dactyl distinctly concave; tufts of setae at mesial base of fixed fingers usually absent but sometimes inconspicuously present, dorsolateral impression at base of propodus weak to moderate; subpalmar tubercles 0–3. Cephalothorax cylindrical or vaulted. Median spine on mesial ramus of uropod reaching or overreaching caudal margin of ramus. Form 1 male with hooks on ischium of third pereopods only; coxa of fourth pereopods lacking large ventral setiferous pit on caudomesial boss; gonopods contiguous at base, distal portion of shaft straight; terminal elements consisting of 1) short, broad, blade-like distally truncate or rounded central projection (occasionally with subapical notch) recurved at about 90° to shaft, 2) swollen mesial process variously shaped and directed, frequently bearing 1–4 small tuberculiform prominences apically, and 3) sometimes with rudimentary caudal knob at caudolateral base of central projection. Females with annulus ventralis subsymmetrical and subrhomboidal or rhomboidal in shape, slightly moveable; first pleopod present, usually reaching or reaching cephalically beyond caudal margin of annulus.

Type-species: *Lacunicambarus diogenes* (Girard, 1852), comb. nov., designated by Hobbs (1969a).

Species included: *Lacunicambarus acanthura* (Hobbs, 1981) comb. nov., *L. diogenes* (Girard, 1852) comb. nov., *L. erythroedactylus* (Simon & Morris, 2015) comb. nov., *L. ludovicianus* (Faxon, 1884) comb. nov., *L. miltus* (Fitzpatrick, 1978) comb. nov., *L. polychromatus* (Thoma, Jezerinac & Simon 2005) comb. nov., and *L. thomai* (Jezerinac, 1993) comb. nov. All of these species were originally assigned to *Cambarus* in the subgenera *Lacunicambarus* or *Tubericanbarus*. The validity and associated character states of *L. erythroedactylus* have been debated and are also under investigation, so this species is omitted from the key below.

Etymology: Hobbs (1969a) coined the name *Lacunicambarus* from the Latin *lacuna* for “cavity” or “pit” and *cambarus* (modified from the Latin *gammarus* for “lobster”). This name is in reference to the fossorial lifestyle of the species in this genus. The gender is masculine.

Key to described species of *Lacunicambarus*

1. Median spine on mesial ramus of uropod overreaching caudal margin of ramus *Lacunicambarus acanthura*
Median spine on mesial ramus of uropod not overreaching caudal margin of ramus 2
2. Dorsomesial margin of palm of chela with two parallel rows of well-developed tubercles, usually numbering 6–8 each, plus a third row running diagonally from mesial base of palm to lateral dactyl articulation, additional scattered tubercles usually present between second and third rows..... 3
Dorsomesial margin of palm of chela with dorsomesial 1/4–1/3 studded with tubercles not forming distinct rows..... 5
3. Cephalic lobe of epistome apically truncate; caudal knob present on gonopod of Form 1 male; life colors include single wide longitudinal stripe on dorsal side of abdomen *Lacunicambarus miltus*
Cephalic lobe of epistome rounded or subtriangular, with or without pointed slender tip; caudal knob absent on gonopod of Form 1 male; life colors do not include longitudinal stripes or include three brightly colored longitudinal stripes on dorsal side of abdomen 4
4. *Rostrum generally narrow with sides strongly concave, forming hourglass shape; central projection of Form 1 male gonopod with well-defined subapical notch; life colors include three brightly colored longitudinal stripes on dorsal side of abdomen *Lacunicambarus ludovicianus*

8.5	Rostrum generally broad with sides straight or weakly concave; central projection of Form 1 male gonopod usually lacking subapical notch; life colors do not include any bright longitudinal striping. <i>Lacunicambarus diogenes</i>	8.75
8.10	5. Rostrum strongly deflected; life color of body greenish, with red or orange highlights and blue hues on the dactyl and distal portion of the chelae <i>Lacunicambarus polychromatus</i>	8.80
8.15	Rostrum weakly deflected; life color of body dark brown to olive, sometimes with orange highlights on rostrum and tips of chelae <i>Lacunicambarus thomai</i>	8.85
8.20	*Because variation across the range of these two species, <i>L. ludovicianus</i> and <i>L. diogenes</i> can be difficult to distinguish using morphology alone. We suggest that life color, particularly striping, be provisionally used as the primary distinguishing character between the species. The utility of color is limited to the identification of live or recently killed specimens as those preserved in ethanol become bleached within days or weeks; however, the taxonomic importance of coloration and color pattern have increasingly been recognized (e.g., Loughman et al., 2015a; Schuster & Taylor, 2016) and is important in this genus.	8.90
8.25		8.95
	<i>Lacunicambarus diogenes</i> (Girard, 1852) comb. nov.	
	(Figs. 4, 5)	
8.30	<i>Astacus fossor</i> Rafinesque, 1817:42.	8.100
8.35	<i>Cambarus diogenes</i> Girard, 1852: 88 [part]; Hay, 1896: 489 [part]; Williamson, 1899: 48 [part]; Ortmann, 1905: 123 [part]; Evermann & Clark, 1920: 719 [part]; Newcombe, 1929: 286 [part]; Creaser, 1931: 267 [part]; Rhoades, 1944a: 146 [part], 1944b: 98 [part]; Eberly, 1955: 283 [part]; Brown, 1955: 62 [part]; Marlow, 1960: 231 [part]; Page, 1985: 434 [part]; Page & Mottes, 1995: 110 [part]; Norrocky, 1989: 67, 1991: 77; Taylor et al., 1996: 25, 2007: 382; 2015: 6; Simon, 2001: 104; Hamr, 2006: 1; Pintor & Soluk, 2006: 58; Welsh & Eversole, 2006: 29; Guiaşu, 2009: 726; Kilian et al., 2010: 11; Loughman, 2010: 218; Stoeckel et al., 2011: 55; Helms et al., 2013: 1333; Morehouse & Tobler, 2013: 101; Simon & Morris, 2015: 572; Peters et al., 2014: 38; Schainost, 2016: 1; Clay et al., 2017: 1177; Glon et al., 2018: 355.	8.105
8.40	<i>Cambarus nebrascensis</i> Girard, 1852: 88 [part]; Faxon, 1885: 75 [part].	8.110
8.45	<i>Cambarus obesus</i> – Hagen, 1870: 81 [part]; Forbes, 1876: 6 [part].	8.115
8.50	<i>Cambarus dubius</i> – Osburn & Williamson, 1898: 21 [part]; Forbes, 1876: 5 [part].	8.120
8.55	<i>Cambarus diogenes</i> Diogenes – Hay, 1899a: 959 [part]; Williamson, 1907: 762 [part]; Marlow, 1960: 233 [part].	8.125
8.60	<i>Cambarus (Bartonius) diogenes</i> – Ortmann, 1906a: 402 [part]; Turner, 1926: 168 [part].	8.130
8.65	<i>Cambarus (Lacunicambarus) diogenes diogenes</i> – Hobbs, 1969b: 110 [part], 1974: 20; [part]; Bouchard, 1972: 56 [part], 1974: 595 [part]; Jezerinac & Thoma, 1984: 123 [part]; Norrocky, 1984: 65; Jezerinac, 1985: 7 [part].	8.135
8.70	<i>Cambarus (Lacunicambarus) diogenes</i> – Jezerinac, 1985: 7 [part]; Hobbs, 1989: 24 [part]; Taylor & Schuster, 2004: 5; Thoma & Armitage, 2008: 1; Breinholt et al., 2012: 3; Hopper & Huryn, 2012: 43; Miller et al. 2014: 15; Crandall & De Grave, 2017: 9.	8.140
8.72	Diagnosis: Eyes pigmented, small. Rostrum curved downwards in lateral view, margins converging, slightly thickened, without marginal spines or tubercles, lacking median carina, shallowly excavated. Acumen distinctly delimited basally by 45° angles. Cephalothorax cylindrical, with 3–5 cervical tubercles. Branchiostegal tubercles inconspicuous, few in number. Suborbital angle acute. Postorbital ridges developed, lacking spine or tubercle. Areola obliterated, constituting, in adults, 39.5–48.7% (mean 42.2%) of entire length of cephalothorax. Antennal scale length 2.4–3.5 (mean 2.8) times	8.142
	width, broadest at midlength, antennal spine strongly developed. Dorsomesial margin of palm of chelae with 3 rows of tubercles, mesial-most row normally consisting of 5–8 (mean 6) tubercles, running parallel to second row with 4–7 (mean 5) tubercles, third row running diagonally from mesial base of palm to lateral dactyl articulation in the form of 0–8 (mean 6) subprobolos tubercles located in shallow dimples. No tufts of elongated setae at mesial base of fixed finger. Opposable margin of dactyl weakly concave at base. Ratio of dactyl length to palm length 1.56–2.2 (mean 1.89). Dorsomedian longitudinal ridges of dactyl, opposable propodus moderately developed. Dorsolateral impression at base of propodus weak. Ventral surface of chelae with 0–3 subpalmar tubercles. Ventral surface of carpus with spine on medial articular rim, mesial margin with 1 or 2 spines. Mesial ramus of uropod with distomedian spine reaching caudal margin, never extending noticeably beyond. Gonopods of Form 1 males contiguous at base, with pronounced umbo near midlength of cephalic surface; terminal elements consisting of short, non-tapering, distally truncate central projection lacking apical notch, approximately equal in length to mesial process, plus mesial process with conical base tipped with protruding finger; both elements directed caudally at angle slightly greater than 90° and just overreaching umbo near midlength of cephalic surface of gonopod. Hooks on ischium of third pereopods only. Female with annulus ventralis rhomboidal, approximately as long as wide, rather deeply embedded in sternum, flexible, with distal half sclerotized and proximal half mildly pliable.	
	Designation of neotype: Girard's type material was destroyed in the Great Chicago Fire of 1871. Faxon (1885) documented the existence of a small number of specimens of various crayfish species labelled with names from Girard (1852), including one labelled “ <i>C. Diogenes</i> ?”, in the Museum of the Academy of Natural Science of Philadelphia (now Academy of Natural Sciences of Drexel University). Faxon (1885), however, did not provide any evidence or explanation tying these specimens to Girard other than the species names on the labels, which he also claimed had been transposed as several specimens did not match their labels. Girard (1852) did not mention the existence of these specimens in his description of <i>L. diogenes</i> . Following the guidelines in ICZN Article 75, we attempted to locate and examine the possible syntypes of <i>L. diogenes</i> by contacting the Academy of Natural Sciences of Drexel University but were told that there were no records of these specimens or of any specimens of <i>L. diogenes</i> matching the correct time period or matching the correct time period or locality (P. Callomon, personal communication). We also inquired at the Muséum national d'Histoire naturelle, Paris about possible syntypes collected by Girard but no specimens of <i>L. diogenes</i> matching the correct time period or locality were located (L. Corbari, personal communication). Given that these mysterious specimens were never mentioned by Girard (1852), are of unclear provenance, and have not been located in over a century, we see no other option than to designate a neotype for <i>L. diogenes</i> , which is described herein.	
	We also include descriptions of particular specimens that we consider as representatives of females and Form 2 males of <i>L. diogenes</i> . These specimens are not type specimens, as the ICZN only permits the designation of a single neotype, but they can be used as reference material if ever needed.	
	Description of neotype, Form 1 male (USNM 1498707): Body subovate (Fig. 4H), cephalothorax height 96% of width. Abdomen narrower than cephalothorax (14.7 and 18.58 mm, respectively; Supplementary material Table S2); maximum width of cephalothorax greater than depth at caudodorsal margin of cervical groove (18.58 and 17.88 mm, respectively). Areola obliterated; length 41.7% of total length of cephalothorax (Fig. 4H). Rostrum curved downwards in lateral view from base to distal end, margins slightly thickened; acumen distinctly delimited basally by 45° angles, anterior tip upturned and not reaching ultimate podomere of antennular peduncle; upper surface of rostrum	

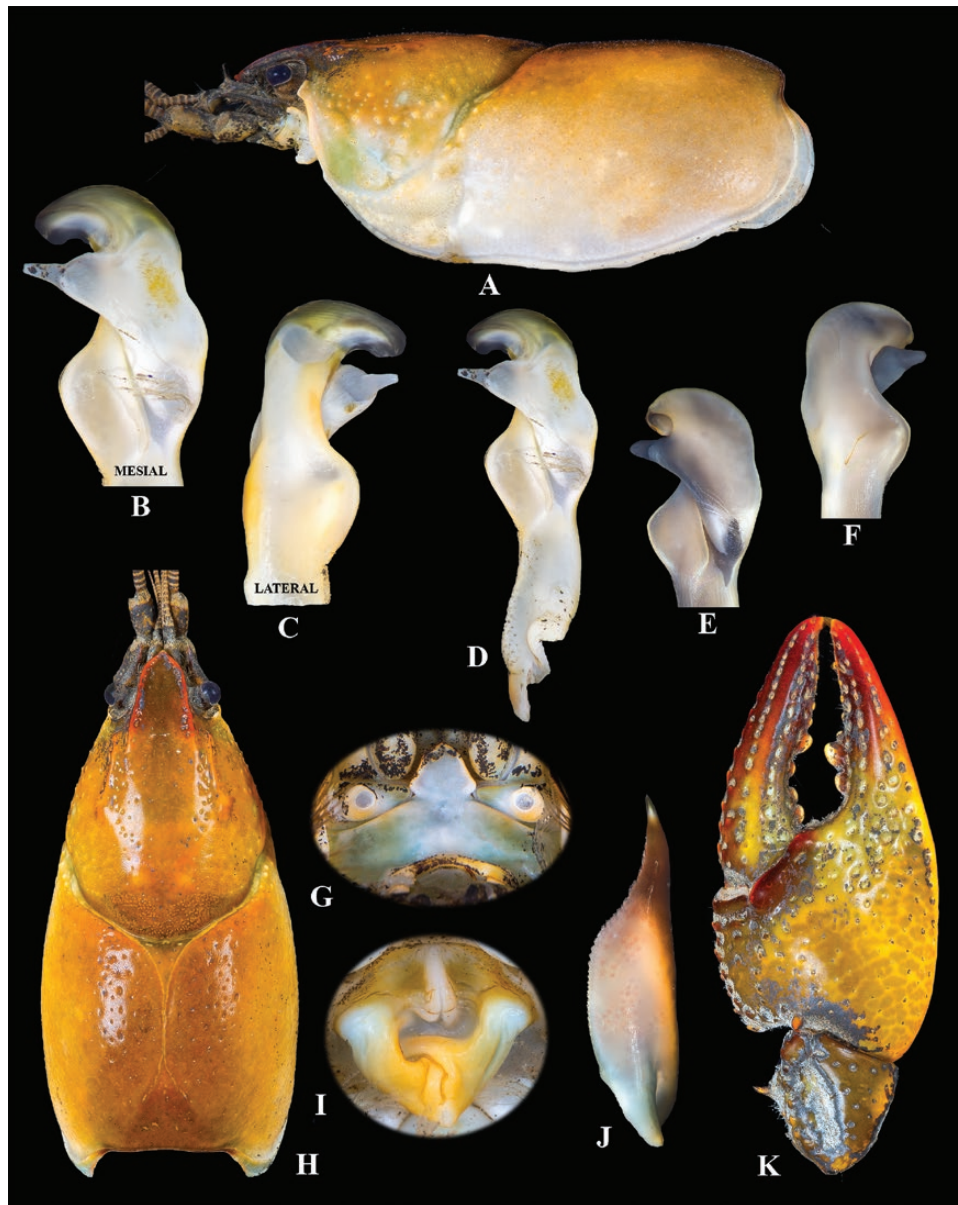


Figure 4. Form 1 neotype male of *Lacunicambarus diogenes* (USNM 1498707) (A–D, G, H, K); representative form 2 male (USNM 1498709) (E, F, J); representative female (USNM 1498708) (L). Lateral view of cephalothorax (A); mesial and lateral views of Form 1 gonopod (B–D); mesial and lateral views of form 2 gonopod (E, F); epistome (G); dorsal view of cephalothorax (H); annulus ventralis (I); dorsal view of antennal scale (J); dorsal view of chela (K). The gonopod is rotated slightly differently in B and D than in C, giving the impression that the length of the central projection differs between these two positions. Photos by G. Schuster

shallowly concave with minute punctations at midrostrum base, forming single row along rostral margins. Subrostral ridge weak but evident in lateral aspect along distal third of rostrum. Postorbital ridges developed, grooved dorsolaterally, ending cephalically without spine or corneous tubercle. Suborbital angle acute; branchiostegal spine replaced by small tubercle. Cervical spines absent, but 5 tubercles present. Hepatic, branchiostegal regions with small tubercles. Remainder of cephalothorax with slight punctations dorsally, laterally. Abdomen subequal in length to cephalothorax, length $2.45\times$ width; pleura short, truncate, rounded caudoventrally. Cephalic section of telson with 2 spines in caudolateral corners, mesial spine moveable. Proximal segment of lateral ramus of uropod with border of 12 spines on distal margin; mesial ramus of uropod with prominent median rib ending distally as strong distomedian spine reaching margin of ramus, laterodistal spine of ramus also strong.

Cephalomedian lobe of epistome (Fig. 4G) short, bell-shaped with uniform raised margins, ventral surface rather flat, lacking setae; main body with shallow foveae; epistomal zygoma moderately arched. Ventral surface of antennular peduncle's proximal podomere without acute spine at base of distal third. Antennular peduncle without spines; antennal scale length $3.2\times$ width (Fig. 4J), broadest at midlength, mesial margin straight at midpoint; antennal spine strong and convex, reaching proximal end of antennular peduncle. Lateral half of ischium of third maxilliped densely studded with long, flexible setae; distolateral angle not acute.

Length of right chela (Fig. 4K) 86.2% cephalothorax length; chela width 49.3% of chela length; palm length 31.8% of chela length; dactyl length $2.07\times$ palm length. Dorsomesial margin of palm of chela with 3 rows of tubercles, mesial-most row composed of 6 tubercles running parallel to second row of 7 tubercles, third row running diagonally from mesial base of palm to

- 10.5 lateral dactyl articulation comprised of 6 subprobolos tubercles located in shallow dimples, 2 distal tubercles between second, third (diagonal) row, dorsal, proximal dorsolateral half smooth, most distolateral area punctate, punctations moderately defined in vicinity of dorsolateral base of propodus; lateral surface of palm, propodus not costate; mesial ventral surface with small punctations, 2 bulbous tubercles on articular rim opposite base of dactyl; 2 subpalmar tubercles. Both fingers of chelae with moderately developed dorsomedian longitudinal ridges; opposable margin of propodus with row of 7 tubercles, decreasing in size except for second from base which is greatly enlarged over adjacent tubercles, seventh from base with corneous tip larger than penultimate tubercle, ventral to single row of denticles. Opposable margin of dactyl with row of 9 tubercles of equal size except for first, fourth from base which are enlarged over adjacent tubercles; single row of minute denticles extending distally from fifth tubercle; mesial surface of dactyl with mesial row of 8 tubercles basally, giving way to punctations distally, second more dorsal row of 3 tubercles. Dorsolateral impression at base of propodus weak.
- 10.10
- 10.15
- 10.20
- 10.25 Cheliped carpus with distinct dorsal furrow; dorsomesial surface with row of 7 tubercles, smaller near distal end; dorsolateral surface with 12 punctations; mesial surface with large procurved spine near distal margin, 3 tubercles triangularly arranged basal to midpoint, with smaller spine located within grouping adjacent to proximal tubercle; ventral surface with spine on distal articular rim. Merus with 3 pre-marginal spines dorsally, row of 5 tubercles on dorsolateral margin, ventrolateral spine, ventromesial row of 9 spines increasing in size from base. Ventral ridge of ischium of first pereopod with small spiniform tubercle. Ischium of third pereopod with simple hook extending proximally over basioischial articulation, not opposed by tubercles on basis. Coxa of fourth pereopod with setiferous, vertically disposed caudomesial boss, ventral surface calcified; coxa of fifth pereopod lacking boss, ventral surface membranous.
- 10.30
- 10.35
- 10.40 Gonopods contiguous at base, reaching coxa of third pereopod; central projection (Fig. 4B–D) short, not tapering, lacking sub-apical notch, curved greater than 90° apically, equal in length to mesial process; mesial process conical at base, then tipped with protruding finger, not directed caudolaterally, directed at 90° to gonopod shaft, caudal knob absent, both terminal elements just overreaching umbo near midlength of cephalic surface of gonopod. Both gonopods of specimen separated from specimen and placed in vial inside specimen jar.
- 10.45
- 10.50 A representative female (USNM 1498708; Fig. 4I) differs from the neotype as follows. Cephalothorax height 108% width. Abdomen wider than cephalothorax (19.67, 19.11 mm, respectively); maximum width of cephalothorax less than depth at caudodorsal margin of cervical groove (19.11, 20.79 mm, respectively); abdomen length 2.1× width. Antennal scale length 2.8× width. Length of right chela 80% cephalothorax length; second, more distal row of tubercles on mesial surface of dactyl with 5 tubercles. Dorsomesial surface of cheliped carpus with row of 6 tubercles; dorsolateral surface of cheliped carpus with 13 punctations. Merus with 2 pre-marginal spines dorsally, ventromesial row of 10 tubercles.
- 10.55
- 10.60 Annulus ventralis (Fig. 4I) rhomboidal, approximately as long as wide, rather deeply embedded in U-shaped sternum, distal half sclerotized, proximal half mildly pliable, with leathery ridge medially located in cephalic half, distal half of ridge bifurcated, ending in central fossa; tongue extending from sclerotized lingual (sinistral) wall into fossa disappearing under sclerotized supralingual (dextral) wall; lingual, supralingual walls asymmetrical, supralingual wall curved on outer margin, lingual wall with primarily straight outer margin; sinus laterally oblong, forming deep fossa on dextral, sinistral sides. Distal margin of annulus ventralis projecting over oblong, asymmetrical (posterior margin higher than anterior margin) post-annular sclerite lacking setae. Setae of dextral first pleopod reaching edge of annulus ventralis when abdomen flexed, setae of sinistral pleopod reaching distal margin of post-annular sclerite.
- 10.65
- 10.70
- 10.72
- A representative Form 2 male (USNM 1498709; Figs. 4E, F, J) differs from the neotype as follows. Left chela regenerated. Dextral antennal scale, sinistral gonopod separated from specimen placed in vial inside specimen jar. Cephalothorax height 108% of width; maximum width of cephalothorax less than depth at caudodorsal margin of cervical groove (19.2, 20.77 mm, respectively). Antennal scale length 3.1× width. Right chela width 44.2% of length; third (diagonal) row of tubercles on dorsomesial margin of palm of chela with 4 subprobolos tubercles located in shallow dimples, 3 distal tubercles positioned between second, third (diagonal) rows; second, more distal row on mesial surface of dactyl with 4 tubercles. Merus with 2 pre-marginal spines dorsally, ventrolateral spine plus proximal spiniform tubercle. Form 2 central projection non-corneous and noticeably shorter than mesial process, just reaching umbo near midlength of cephalic surface of gonopod. Mesial process retains conical base plus finger-like projection.
- 10.75
- 10.80
- 10.85
- 10.90
- 10.95
- 10.100
- Type locality:* Girard (1852) did not designate a type locality for *L. diogenes* but mentioned finding the species “in the neighborhoods of the city of Washington” (i.e., Washington, DC; Girard, 1852: 89). Grow & Merchant (1980) and Grow (1981, 1982) studied a population of *L. diogenes* on Theodore Roosevelt Island in the Potomac River within the boundaries of Washington, DC. We collected the neotype, representative female, and representative Form 2 male on the southwestern side of this island, between the footbridge connecting the parking lot to the island and Theodore Roosevelt Bridge (38.893127° N, –77.062733° W). We collected the specimens under the National Park Service Scientific Research and Collecting Permit GWMP-2018-SCI-0003.
- 10.105
- 10.110
- Geographical distribution:* *Lacunicambarus diogenes* represents either a widely-distributed species or several narrowly-distributed, undescribed species as suggested by Faxon (1885), Ortmann (1906a), Hobbs (1989), and Jezerinac (1993). Our results support the latter hypothesis, and we accordingly regard the range of *L. diogenes* sensu stricto as being restricted to eastern North America. The species has been documented in the Atlantic Coastal Plain and parts of the Piedmont ecoregion from New Jersey, Pennsylvania, Delaware, Maryland, Virginia, North Carolina, South Carolina, and Georgia (Faxon, 1885; Hobbs, 1981; Jezerinac, 1993; Taylor et al., 2007).
- 10.115
- 10.120
- 10.125
- 10.130
- 10.135
- Hobbs (1981) observed a geographic break in the distribution of *L. diogenes* sensu lato in Georgia, with specimens from northeastern Georgia exhibiting the coloration typical of *L. diogenes* sensu stricto (see color notes and Fig. 5) and those from western Georgia exhibiting the coloration that Hobbs associated with specimens from Alabama, Tennessee, and Mississippi. We did not include specimens from Georgia in our analyses, but we included a number of specimens from the eastern and Gulf of Mexico coasts of North America and found them to form distinct clades (Fig. 2), a pattern that has been documented in marine, terrestrial, and freshwater species, including in the crayfish *Creaserinus fodiens* (Cottle, 1863) (Hobbs, 1989; Soltis et al., 2006). Our east Coast specimens grouped with specimens from the type locality of *L. diogenes* in Washington, D.C., whereas the specimens from the Gulf of Mexico coast grouped with specimens from the type locality of *L. erythrodactylus* in Alabama. This suggests that the specimens of *Lacunicambarus* from the Gulf of Mexico are distinct from *L. diogenes* and will need to be elevated to the species level. Such change will require a review of the taxonomic validity of the Alabama species (*L. erythrodactylus*), whose description has proven puzzling. Until further revisionary work on *Lacunicambarus* is completed, we recommend that all undescribed *Lacunicambarus* specimens that resemble *L. diogenes* but that fall outside of the geographic range outlined above be provisionally referred to as *Lacunicambarus* aff. *diogenes*.
- 10.140
- 10.142
- Specimens examined:* Supplementary material Table S3 gives a complete list of the specimens of *L. diogenes* examined and their collection localities.
- Conservation status:* *Lacunicambarus diogenes* is fairly common in its preferred habitat and is tolerant of a certain amount of anthropogenic



Figure 5. Dorsal view of Form 1 neotype male of *Lacunicambarus diogenes* (USNM 1498707). Photo by G. Schuster.

disturbance, as evidenced by its propensity to burrow in roadside drainage ditches. Reductions in the range of this species have been recorded and are tentatively attributed to urbanization and biological invasions by crayfishes such as the red swamp crayfish, *Procambarus clarkii* (Girard, 1852) (Glon *et al.*, 2018). *Lacunicambarus diogenes* has been classified as Currently Stable by the American Fisheries Society's Endangered Species Crayfish Subcommittee (Taylor *et al.*, 2007) and of Least Concern by the IUCN (IUCN, 2001). We suggest that additional sampling be periodically conducted to assess the conservation status of this species, with a special emphasis on assessing threats from habitat loss and invasive species.

Color notes: The color in the original description of *L. diogenes* is given as "body uniform yellowish brown above and below, greenish on the sides and on the claws, the tips of which are red" (Girard, 1852: 89). We concur with this account but supplement it as follows. The neotype exhibits the orangish-brown or bourbon color that Girard noted on its cephalothorax and chelae (Fig. 5), but we examined specimens from the type locality and surrounding areas exhibiting a light brown or tobacco colored cephalothorax. Some specimens from the type locality had olive-green instead of yellowish-brown chelae, but still bearing red highlights. These red or burgundy highlights are found not only on the tips of the claws but also on the rostrum, post-orbital ridges, and on the distal margin of its abdominal somites and uropods. Other than the red or burgundy highlights, the abdomen is typically darker and drabber than the body, which is often coffee brown. The ventral portion of the cephalothorax and the base of the pereopods are white or off-white, although this may only be evident in freshly molted or cleaned specimens (Fig. 4A).

Variation: In the original description of *L. diogenes*, Girard (1852) identified only three morphological characteristics (length and shape of the rostrum and width of the areola) but wrote several paragraphs on

the ecology of the species, describing its burrowing habits in detail. This has resulted in the lumping of numerous populations of burrowing crayfishes from throughout North America under this species epithet without considering morphological characters. As a result, *L. diogenes* sensu lato has historically been considered one of the most widely distributed crayfishes in North America, found in nearly every state east of the Rocky Mountains and in Ontario, Canada. This is unfortunate because it obscures any local variation in morphological traits, which could result in inaccurate conservation assessments when all of these populations are considered to be homogeneous.

Lacunicambarus diogenes sensu stricto exhibits a moderate amount of variation across its range beyond the color variation mentioned above and normal ontogenic variation (i.e., juveniles having less developed features such as chelae sculpturing, tubercles, and spines). Specimens from populations in the northern part of the range of the species were observed with a noticeably concave rostrum and a cephalothorax covered laterally with granular tubercles. Specimens from populations in the southern part of the range had remarkably spinose chelipeds. We also observed throughout the range variation in the composition of the third (diagonal) row of tubercles on the dorsomesial margin of the palms of the chelae ranging from punctations that are nearly indiscernible to prominent tubercles that are not subproboles, as well as variation in the curvature of the spines of the antennal scales ranging from straight to curving outwards rather than inwards.

Size: *Lacunicambarus diogenes* is a moderately sized crayfish, particularly when compared to congeners, which can reach 70 mm in carapace length (CL). The largest specimen we examined was a female from Virginia that measured 46.76 mm CL.

Life History: The life history of *L. diogenes* has been thoroughly covered by Girard (1852), Grow & Merchant (1980), and Grow (1981, 1982). The crayfish is a primary burrower that preferentially burrows in clay soils in wet meadows and floodplains. Individuals can sometimes be found in vernal pools outside of burrows in early Spring.

Crayfish associates: Given the large range and habitat preference of *L. diogenes*, the species can be found in association with all primary and secondary burrowing crayfishes occurring in the Atlantic Coastal Plain and Piedmont ecoregions. Hobbs (1981) reported collecting the following species either in burrows adjacent to those of *L. diogenes* or in nearby streams in Georgia: *Cambarus latimanus* (Le Conte, 1856), *C. reflexus* Hobbs, 1981, *C. striatus* Hay, 1902, *Creaserinus fodiens*, *Faxonella clypeata* (Hay, 1899b), *Procambarus acutissimus* (Girard, 1852), *P. enoplosternum* Hobbs, 1947, *P. pubescens* (Faxon, 1884), *P. gibbus* Hobbs, 1969c, *P. spiculifer* (Le Conte, 1856), *P. howellae* Hobbs, 1952, *P. paeninsulanus* (Faxon, 1914), and *P. troglodytes* (Le Conte, 1856). Because Hobbs (1981) considered specimens from northeastern and western Georgia to belong to a single species, it is unclear whether these crayfish associations are with *L. diogenes* or *L. aff. diogenes* from the Gulf of Mexico. Glon *et al.* (2018) reported finding populations of *P. acutus* (Girard, 1852) and *P. clarkii* at sites that had historically been occupied by *L. diogenes* in eastern Philadelphia but did not find either of these species occurring in sympatry with *L. diogenes*. *Lacunicambarus diogenes* is not known to occur in sympatry with any congeners.

Etymology: We speculate that Girard chose the name *diogenes* for the Greek philosopher Diogenes, who is often portrayed living in a wine jar or barrel. Girard possibly found the respective abodes of Diogenes and of the crayfish analogous.

SUPPLEMENTARY MATERIAL

Supplementary material Table S1. List of specimens used in molecular phylogenetic analyses.

Supplementary material Table S2. Measurements of neotype and representative female and Form 2 specimens of *Lacunicambarus diogenes*.

- 12.5 Supplementary material Table S3. List of specimens of *Lacunicambarus diogenes* examined.

ACKNOWLEDGEMENTS

- 12.10 We thank Z. Barnett, R. Bearden, C. Bonvillain, J. Buckley, C. Davis, D. Foltz, H. Glon, B. Gregory, P. Hamr, D. Horton, D. Johnson, R. Lemaître, D. Lieb, Z. Loughman, S. McGregor, A. Mularo, G. Myers, K. Reed, S. Schainost, K. Scott, J. Stoeckel, A. Sykes, B. Williams, C. Williams, N. Wood, and the Crayfish Conservation Laboratory of West Liberty University for help to procure samples; K. Crandall and D. Stern for advice on DNA sequencing; G. Schuster for help with photography and the preparation of figures, and S. Smith for help with logistics. Partial funding was obtained from The Ohio State University, as well as a grant from United States Fish and Wildlife Service South East at Risk Species awarded to Z. Loughman and B. Williams, the Midwest Biodiversity Institute, and Friends of the North Carolina Museum of Natural Sciences. We also thank two anonymous reviewers and the editors for their help in preparing the manuscript.

REFERENCES

- 12.30 Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K., Meier, R., Winker, K., Ingram, K.K. & Das, I. 2007. Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, **22**: 148–155.
- Bland, L.M. 2017. Global correlates of extinction risk in freshwater crayfish. *Animal Conservation*, **20**: 532–542.
- 12.35 Bouchard, R.W. 1972. *A contribution to the knowledge of Tennessee crayfish*. Ph.D., University of Tennessee, Knoxville, TN, USA.
- Bouchard, R.W. 1974. Crayfishes of the Nashville Basin, Tennessee, Alabama, and Kentucky (Decapoda, Astacidae). *Association of Southeastern Biologists Bulletin*, **21**: 41.
- 12.40 Bouchard, R. W. & Bouchard, J. W. 1995 Two new species and subgenera (*Cambarus* and *Orconectes*) of crayfishes (Decapoda: Cambaridae) from the eastern United States. *Notulae Naturae*, **471**: 1–21.
- Bouchard, R.W. & Etnier, D.A. 1979. A new species of primary burrowing crayfish of the genus *Cambarus* from the Ridge and Valley Province in Tennessee. *Proceedings of the Biological Society of Washington*, **92**: 589–600.
- 12.45 Bott, R. 1950. Die Flußkrebse Europas (Decapoda, Astacidae). *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*, **483**: 1–36, pls. 1–6.
- Breinholt, J.W., Porter, M.L. & Crandall, K.A. 2012. Testing phylogenetic hypotheses of the subgenera of the freshwater crayfish genus *Cambarus* (Decapoda: Cambaridae). *PLoS One*, **7**: e46105.
- 12.50 Brown, P.L. 1955. The biology of the crayfishes of central and southeastern Illinois. Ph.D. thesis, University of Illinois, Urbana-Champaign, IL.
- Buhay, J.E. & Crandall, K.A. 2005. Subterranean phylogeography of freshwater crayfishes shows extensive gene flow and surprisingly large population sizes. *Molecular Ecology*, **14**: 4259–4273.
- 12.55 Chernomor, O., von Haeseler, A. & Minh, B.Q. 2016. Terrace aware data structure for phylogenomic inference from supermatrices. *Systematic Biology*, **65**: 997–1008.
- Clay, M., Stoeckel, J. & Helms, B. 2017. The role of abiotic and biotic cues in burrow habitat selection by juvenile crayfish. *Behaviour*, **154**: 1177–1196.
- 12.60 Cope, E.D. 1872. On the Wyandotte Cave and its fauna. *American Naturalist*, **6**: 406–422.
- Cottle, T.J. 1863. On the two species of *Astacus* found in Upper Canada. *Canadian Journal of Industry, Science, and Art*, n. ser., **8**: 216–219.
- Crandall, K.A. & De Grave, S. 2017. An updated classification of the freshwater crayfishes (Decapoda: Astacidea) of the world, with a complete species list. *Journal of Crustacean Biology*, **37**: 615–653.
- 12.65 Crandall, K.A. & Fitzpatrick Jr, J.F. 1996. Crayfish molecular systematics: using a combination of procedures to estimate phylogeny. *Systematic Biology*, **45**: 1–26.
- Crandall, K.A., Robison, H.W. & Buhay, J.E. 2008. Avoidance of extinction through nonexistence: the use of museum specimens and molecular genetics to determine the taxonomic status of an endangered freshwater crayfish. *Conservation Genetics*, **10**: 177–189.
- 12.70 Creaser, E.P. 1931. The Michigan decapod crustaceans. *Michigan Academy of Science, Arts and Letters*, **13**: 257–276.

- 12.75 Creaser, E.P. 1933. Descriptions of some new and poorly known species of North American crayfishes. *Occasional Papers of the Museum of Zoology, University of Michigan*, **275**: 1–21, pls. 1, 2.
- Darriba, D., Taboada, G.L., Doallo, R. & Posada, D. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, **9**: 772.
- 12.80 Dubois, A. 2003. The relationships between taxonomy and conservation biology in the century of extinctions. *Comptes Rendus Biologies*, **326**: 9–21.
- Eberly, W.R. 1955. Summary of the distributions of Indiana crayfishes, including new state and county records. *Proceedings of the Indiana Academy of Science*, **64**: 281–283.
- 12.85 Edgar, R.C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research*, **32**: 1792–1797.
- Erichson, W.F. 1846. Uebersicht der arten der gattung *Astacus*. *Archiv für Naturgeschichte*, **12**: 86–103.
- 12.90 Evermann, B.W. & Clark, H.W. 1920. *Lake Maxinkuckee: a physical and biological survey*. Indiana Department of Conservation Publication, Indianapolis, IN, USA.
- Fabricius, J.C. 1775. *Systema Entomologiae, sistens Insectorum classes, ordines, genera, species, adiectis synonymis, locis, descriptionibus, observationibus*. Flensbergi et Lipsiae [= Leipzig].
- 12.95 Fabricius, J.C. 1798. *Supplementum Entomologiae Systematicae*. Proft et Storch, Hafniae [= Copenhagen].
- Faxon, W. 1884. Descriptions of new species of *Cambarus*, to which is added a synonymical list of the known species of *Cambarus* and *Astacus*. *Proceedings of the American Academy of Arts and Sciences*, **20**: 107–158.
- 12.100 Faxon, W. 1885. A revision of the Astacidae Part I. The genera *Cambarus* and *Astacus*. *Memoirs of the Museum of Comparative Zoology at Harvard College*, **10**: 1–186.
- Faxon, W. 1914. Notes on the crayfishes in the United States National Museum and the Museum of Comparative Zoology with descriptions of new species and subspecies to which is appended a catalogue of the known species and subspecies. *Memoirs of the Museum of Comparative Zoology at Harvard College*, **40**: 352–427, pls. 1–13.
- 12.105 Fitzpatrick, J.F. Jr. 1978. A new burrowing crawfish of the genus *Cambarus* from Southwest Alabama (Decapoda, Cambaridae). *Proceedings of the Biological Society of Washington*, **91**: 748–755.
- 12.110 Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**: 294–299.
- Forbes, S.A. 1876. List of Illinois Crustacea, with descriptions of new species. *Illinois Museum of Natural History*, **1**: 3–25.
- 12.115 Girard, C. 1852. A revision of the North American Astaci, with observations on their habits and geographical distribution. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **6**: 87–91.
- Glon, M.G., Mularo, A.J., Lieb, D.A. & Loughman, Z.J. 2018. Rediscovery of *Cambarus diogenes* (Devil Crayfish) in Pennsylvania, USA. *Northeastern Naturalist*, **25**: 355–361.
- 12.120 Grow, L. 1981. Burrowing behaviour in the crayfish *Cambarus diogenes diogenes* Girard. *Animal Behaviour*, **29**: 351–356.
- Grow, L. 1982. Burrowing/soil-texture relationships in the crayfish, *Cambarus diogenes diogenes* Girard (Decapoda, Astacidea). *Crustaceana*, **42**: 150–157.
- 12.125 Grow, L. & Merchant, H. 1980. The burrow habitat of the crayfish, *Cambarus diogenes diogenes* (Girard). *American Midland Naturalist*, **103**: 231–237.
- Guiaşu, R.C. 2009. Conservation, status, and diversity of the crayfishes of the genus *Cambarus* Erichson, 1846 (Decapoda, Cambaridae). *Crustaceana*, **82**: 721–742.
- 12.130 Guindon, S. & Gascuel, O. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. *Systematic Biology*, **52**: 696–704.
- Hamr, P. 2006. The distribution and conservation status of burrowing crayfishes *Fallicambarus fodiens* and *Cambarus diogenes* in Canada. *Freshwater Crayfish*, **15**: 271–282.
- 12.135 Hay, W.P. 1896. The Crawfishes of the State of Indiana. *20th Annual Report of the Department of Geology and Natural Resources of Indiana*, pp. 476–506.
- Hay, W.P. 1899a. Synopsis of North American invertebrates, VI: the Astacidae of North America. *American Naturalist*, **33**: 957–966.
- 12.140 Hay, W.P. 1899b. Description of two new species of crayfish. *Proceedings of the United States National Museum*, **22**: 121–124.
- 12.142 Hay, W.P. 1902. Observations on the crustacean fauna of Nickajack Cave, Tennessee, and vicinity. *Proceedings of the United States National Museum*, **25**: 417–439.

- 13.5 Helms, B., Budnick, W., Pecora, P., Skipper, J., Kosnicki, E., Feminella, J. & Stoeckel, J. 2013. The influence of soil type, congeneric cues, and floodplain connectivity on the local distribution of the devil crayfish (*Cambarus diogenes* Girard). *Freshwater Science*, **32**: 1333–1344.
- 13.10 Hobbs, H.H. Jr. 1947. Two new crayfishes of the genus *Procambarus* from Georgia, with notes on *Procambarus pubescens* (Faxon). *Quarterly Journal of the Florida Academy of Sciences*, **9**: 1–18.
- Hobbs, H.H. Jr. 1952. A new crayfish of the genus *Procambarus* from Georgia with a key to the species of the *clarkii* subgroup. *Quarterly Journal of the Florida Academy of Sciences*, **15**: 165–173.
- 13.15 Hobbs, H.H. Jr. 1969a. On the distribution and phylogeny of the crayfish genus *Cambarus*. In: *The distributional history of the biota of the southern Appalachians* (P.C. Holt, ed.), pp. 93–178. Virginia Polytechnic Institute, Blacksburg, VA.
- Hobbs, H.H. Jr. 1969b. A checklist of the North and Middle American crayfishes (Decapoda: Astacidae and Cambaridae). *Smithsonian Contributions to Zoology*, **166**.
- 13.20 Hobbs, H.H. Jr. 1969c. Two new species of the crayfish genus *Procambarus* (Decapoda, Astacidae) with keys to the members of the *spiculifer* group. *Proceedings of the Biological Society of Washington*, **82**: 329–348.
- Hobbs, H.H. Jr. 1970. A new crayfish from the Nashville Basin, Tennessee. *Proceedings of the Biological Society of Washington*, **83**: 161–170.
- 13.25 Hobbs, H.H. Jr. 1973. New species and relationships of the members of the genus *Fallicambarus*. *Proceedings of the Biological Society of Washington*, **86**: 461–482.
- Hobbs, H.H. Jr. 1974. A checklist of the North and Middle American crayfishes (Decapoda: Astacidae and Cambaridae). *Smithsonian Contributions to Zoology*, **166**: 1–161.
- 13.30 Hobbs, H.H. Jr. 1981. The crayfishes of Georgia. *Smithsonian Contributions to Zoology*, **318**: 1–549.
- Hobbs, H.H. Jr. 1989. An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). *Smithsonian Contributions to Zoology*, **480**: 1–244.
- 13.35 Hobbs, H.H. Jr. & Robison, W. 1989. On the crayfish genus *Fallicambarus* (Decapoda, Cambaridae) in Arkansas, with notes on the *fodiens* complex and descriptions of 2 new species. *Proceedings of the Biological Society of Washington*, **102**: 651–697.
- Hopper, J.D. & Hurny, A.D. 2012. A new, non-destructive method for sampling burrowing crayfish. *Southeastern Naturalist*, **11**: 43–48.
- 13.40 Huelsenbeck, J.P. & Ronquist, F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**: 754–755.
- ICZN (International Commission on Zoological Nomenclature). 1999. International code of zoological nomenclature, Edn. 4. International Trust for Zoological Nomenclature and British Museum, London.
- 13.45 Jezerinac, R.F. 1985. On the two forms of *Cambarus (Lacunicambarus) diogenes* (Decapoda: Cambaridae) in Ohio. *Ohio Journal of Science*, **85**: 7.
- Jezerinac, R.F. 1993. A new subgenus and species of crayfish (Decapoda: Cambaridae) of the genus *Cambarus*, with an amended description of the subgenus *Lacunicambarus*. *Proceedings of the Biological Society of Washington*, **106**: 532–544.
- 13.50 Jezerinac, R.F. & Thoma, R.F. 1984. An illustrated key to Ohio *Cambarus* and *Fallicambarus* (Decapoda: Cambaridae), with comments and a new subspecies record. *Ohio Journal of Science*, **84**: 120–125.
- Johnson, G.T., Elder, J.F. Jr, Thompson, S.M. & Hightower, P. 2011. Phylogeny of the freshwater crayfish subfamily Cambarinae based on 16S rDNA gene analysis. *Current Trends in Ecology*, **2**: 97–113.
- 13.55 Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Mentjies, P. & Drummond, A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, **28**: 1647–1649.
- 13.60 Kilian, J.V., Becker, A.J., Stranko, S.A., Ashton, M., Klauda, R.J., Gerber, J. & Hurd, M. 2010. The status and distribution of Maryland crayfishes. *Southeastern Naturalist*, **9**: 11–32.
- Le Conte, J. 1856. Descriptions of new species of *Astacus* from Georgia. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **7**: 400–402.
- 13.65 Loughman, Z.J. 2010. Ecology of *Cambarus dubius* (upland burrowing crayfish) in north-central West Virginia. *Southeastern Naturalist*, **9**: 217–230.
- Loughman, Z. J. & Fetzner Jr., W. 2015. Astacology and crayfish conservation in the southeastern United States: Past, present and future. *Freshwater Crayfish*, **21**: 1–5.
- 13.70 Loughman, Z. J., Lieb, D.A., Scott, R.K., Dillard, Z.W. & Sadecky, N.M. 2017a. Historical and current distribution of Appalachian primary burrowing crayfishes (Decapoda: Astacoidea: Cambaridae) in western Pennsylvania: a century of change or stasis? *Journal of Crustacean Biology*, **37**: 535–543.
- Loughman, Z. J., Thoma, R. F., Fetzner Jr, J. W., & Stocker, G. W. 2015a. *Cambarus (Jugicambarus) pauleyi*, a new species of crayfish (Decapoda: Cambaridae) endemic to southcentral West Virginia, USA, with a re-description of *Cambarus (J.) dubius*. *Zootaxa*, **3980**: 526–546.
- 13.80 Loughman, Z. J., Welsh, S.A., Fetzner Jr, J.W. & Thoma, R.F. 2015b. Conservation of imperiled crayfish, *Cambarus veteranus* (Decapoda: Reptantia: Cambaridae). *Journal of Crustacean Biology*, **35**: 850–860.
- Loughman, Z. J., Welsh, S.A., Sadecky, N. M., Dillard, Z. W., Scott, R. K. 2017b. Evaluation of physicochemical and physical habitat associations for *Cambarus callainus* (Big Sandy crayfish), an imperiled crayfish endemic to the Central Appalachians. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **27**: 755–763.
- 13.85 Marlow, G. 1960. The subspecies of *Cambarus diogenes*. *American Midland Naturalist*, **64**: 229–250.
- Miller, J.M., Niraula, B.B., Reátegui-Zirena, E.G. & Stewart, P.M. 2014. Life history and physical observations of primary burrowing crayfish (Decapoda: Cambaridae) *Cambarus (Lacunicambarus) diogenes* and *Cambarus (Tubericambarus) polychromatus*. *Journal of Crustacean Biology*, **34**: 15–24.
- 13.90 Mokady, O., Rozenblatt, S., Graur, D. & Loya, Y. 1994. Coral-host specificity of Red Sea *Lithophaga* bivalves: interspecific and intraspecific variation in 12S mitochondrial ribosomal RNA. *Molecular Marine Biology and Biotechnology*, **3**: 158–164.
- Morehouse, R.L. & Tobler, M. 2013. Crayfishes (Decapoda: Cambaridae) of Oklahoma: identification, distributions, and natural history. *Zootaxa*, **3717**: 101–157.
- 13.95 Newcombe, C.L. 1929. The crayfishes of West Virginia. *Ohio Journal of Science*, **29**: 267–288.
- Nguyen, L.T., Schmidt, H.A., von Haeseler, A. & Minh, B.Q. 2014. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, **32**: 268–274.
- 13.100 Norrocky, M.J. 1984. Burrowing crayfish trap. *Ohio Journal of Science*, **84**: 65–66.
- Norrocky, M.J. 1989. New distribution records for the crayfish *Cambarus (Cambarus) ortmanni* Williamson (Decapoda: Cambaridae) with life history notes. *Ohio Journal of Science*, **89**: 67–69.
- 13.110 Norrocky, M.K. 1991. Observations on the ecology, reproduction, and growth of the burrowing crayfish *Fallicambarus (Creaserinus) fodiens* (Decapoda: Cambaridae) in North-central Ohio. *American Midland Naturalist*, **125**: 75–86.
- Ortmann, A.E. 1905. The mutual affinities of the species of the genus *Cambarus*, and their dispersal over the United States. *Proceedings of the American Philosophical Society*, **44**: 91–136.
- 13.115 Ortmann, A.E. 1906a. The crawfishes of the State of Pennsylvania. *Memoirs of the Carnegie Museum*, **2**: 343–524.
- Ortmann, A.E. 1906b. A case of isolation without barriers. *Science*, **23**: 504–506.
- 13.120 Osburn, R.C. & Williamson, E.B. 1898. Crayfishes of Ohio. 6th Annual Report of the Ohio Academy of Science, pp 1–21. Ohio Academy of Science, Columbus, OH, USA.
- Owen, C.L., Bracken-Grissom, H., Stern, D. & Crandall, K. A. 2015. A synthetic phylogeny of freshwater crayfish: insights for conservation. *Philosophical Transactions of the Royal Society of London B*, **370**: 1–8.
- 13.125 Page, L.M. 1985. The crayfishes and shrimps (Decapoda) of Illinois. *Illinois Natural History Survey Bulletin*, **33**: 335–448.
- Page, L.M. & Mottesi, G.B. 1995. The distribution and status of the Indiana crayfish, *Orconectes indianensis*, with comments on the crayfishes of Indiana. *Proceedings of the Indiana Academy of Science*, **104**: 103–111.
- 13.130 Peters, J.A., Cooper, M.J., Creque, S.M., Kornis, M.S., Maxted, J.T., Perry, W.L., Schueler, F.W., Simon, T.P., Taylor, C.A., Thoma, R.F., Uzarski, D.G. & Lodge, D.M. 2014. Historical changes and current status of crayfish diversity and distribution in the Laurentian Great Lakes. *Journal of Great Lakes Research*, **40**: 35–46.
- Pintor, L.M. & Soluk, D.A. 2006. Evaluating the non-consumptive, positive effects of a predator in the persistence of an endangered species. *Biological Conservation*, **130**: 584–591.
- 13.135 Rafinesque, C.S. 1817. Synopsis of four new genera and ten new species of Crustacea, found in the United States. *American Monthly Magazine and Critical Review*, **2**: 41–44.
- Rhoades, R.R. 1944a. The crayfishes of Kentucky, with notes on variation, distribution, and descriptions of new species and subspecies. *American Midland Naturalist*, **31**: 111–149.
- 13.140 Rhoades, R.R. 1944b. The crayfishes of Kentucky, with notes on variation, distribution, and descriptions of new species and subspecies. *American Midland Naturalist*, **31**: 111–149.
- 13.142

- 14.5 Rhoades, R.R. 1944b. Further studies on distribution and taxonomy of Ohio crayfish, and the description of a new subspecies. *Ohio Journal of Science*, **44**: 96–99.
- Richly, E. & Leister, D. 2004. NUMTs in sequenced eukaryotic genomes. *Molecular Biology and Evolution*, **21**: 1081–1084.
- 14.10 Richman, N.I., Bohm, M., Adams, S.B., Alvarez, F., Bergey, E.A., Bunn, J.J., Burnham, Q., Cordeiro, J., Coughran, J., Crandall, K.A., Dawkins, K.L., DiStefano, R.J., Doran, N.E., Edsman, L., Eversole, A.G., Fureder, L., Furse, J.M., Gherardi, F., Hamr, P., Holdich, D.M., Horwitz, P., Johnston, K., Jones, C.M., Jones, J.P., Jones, R.L., Jones, T.G., Kawai, T., Lawler, S., López-Mejía, M., Miller, R.M., Pedraza-Lara, C., Reynolds, J.D., Richardson, A.M., Schultz, M.B., Schuster, G.A., Sibley, P.J., Souty-Grosset, C., Taylor, C.A., Thoma, R.F., Walls, J., Walsh, T.S. & Collen, B. 2015. Multiple drivers of decline in the global status of freshwater crayfish (Decapoda: Astacidea). *Philosophical Transactions of the Royal Society of London B*, **370**: 20140060 [doi: 10.1098/rstb.2014.0060].
- 14.15 Ronquist, F. & Huelsenbeck, J. P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**: 1572–1574.
- Schainost, S.C. 2016. *The crayfish of Nebraska*. Nebraska Game and Parks Commission, Lincoln, NE, USA.
- 14.25 Schuster, G. A., & Taylor, C. A. 2016. *Cambarus (Depressicambarus) clairitae*, a new species of crayfish (Decapoda: Cambaridae) from Alabama with a review of the *halli* group in the subgenus *Depressicambarus*. *Zootaxa*, **4193**: 332–346.
- Simon, T.P. 2001. A checklist of the crayfish and freshwater shrimp (Decapoda) of Indiana. *Proceedings of the Indiana Academy of Science*, **110**: 104–110.
- 14.30 Simon, T.P. & Morris, C.C. 2015. *Cambarus (Lacunicambarus) erythrodictylus*, a new species of crayfish (Decapoda: Cambaridae) of the *Cambarus diogenes* complex from Alabama and Mississippi, USA. *Proceedings of the Biological Society of Washington*, **127**: 572–584.
- Soltis, P.S. & Gitzendanner, M.A. 1999. Molecular systematics and the conservation of rare species. *Conservation Biology*, **13**: 471–483.
- 14.35 Soltis, D.E., Morris, A.B., McLachlan, J.S., Manos, P.S. & Soltis, P.S. 2006. Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology*, **15**: 4261–4293.
- Stoeckel, J.A., Helms, B.S. & Cash, E. 2011. Evaluation of a crayfish burrowing chamber design with simulated groundwater flow. *Journal of Crustacean Biology*, **31**: 50–58.
- 14.40 Taylor, C.A. & Schuster, G.A. 2004. The crayfishes of Kentucky. *Illinois Natural History Survey Special Publication*, **28**: 1–220.
- Taylor, C.A. & Knouft, J.H. 2006. Historical influences on genital morphology among sympatric species: gonopod evolution and reproductive isolation in the crayfish genus *Orconectes* (Cambaridae). *Biological Journal of the Linnean Society*, **89**: 1–12.
- 14.80 Taylor, C.A., Schuster, G.A., Cooper, J.E., DiStefano, R.J., Eversole, A.G., Hamr, P., Hobbs, H.H. III, Robison, H.W., Skelton, C.E. & Thoma, R.F. 2007. A reassessment of the conservation status of crayfishes of the United States and Canada after 10+ years of increased awareness. *Fisheries*, **32**: 372–389.
- Taylor, C.A., Schuster, G.A. & Wylie, D.B. 2015. Field guide to crayfishes of the Midwest. *Illinois Natural History Survey Manual*, **15**: 1–145.
- 14.85 Taylor, C.A., Warren, M.L. Jr, Fitzpatrick, J.F., Hobbs, H.H. III, Jezerinac, R.F., Pflieger, W.L. & Robison, H.W. 1996. Conservation status of crayfishes of the United States and Canada. *Fisheries*, **21**: 25–38.
- Thoma, R.F. 2011. *Cambarus (Tubericambarus) stockeri* (Decapoda: Cambaridae) a new species of plesiomorphic *Cambarus* from Georgia and Tennessee with zoogeographic affinity to *Cambarus (Depressicambatus) cymatilis*. *Proceedings of the Biological Society of Washington*, **124**: 318–325.
- 14.90 Thoma, R.F. & Armitage, B.J. 2008. *Burrowing crayfish of Indiana*. Final Report to the Indiana Department of Natural Resources, Indianapolis, IN, USA.
- Thoma, R.F., Jezerinac, R.F. & Simon, T.P. 2005. *Cambarus (Tubericambarus) polychromatus* (Decapoda: Cambaridae), a new species of crayfish from the United States. *Proceedings of the Biological Society of Washington*, **118**: 326–336.
- 14.95 Turner, C.L. 1926. A new method of investigating the hydrotropisms of freshwater invertebrates. *Biological Bulletin*, **46**: 35–54.
- U.S. Fish and Wildlife Service. 2016. Threatened species status for the Big Sandy Crayfish and Endangered Species Status for the Guyandotte River Crayfish. Federal Register, **Vol. 81**, pp. 20450–20481, Washington, DC [https://www.gpo.gov/fdsys/pkg/FR-2016-04-07/pdf/2016-07744.pdf].
- Welsh, S.M. & Eversole, A.G. 2006. Comparison of two burrowing crayfish trapping methods. *Southeastern Naturalist*, **5**: 27–30.
- 14.105 Wheeler, Q.D. 2004. Taxonomic triage and the poverty of phylogeny. *Philosophical Transactions of the Royal Society of London B*, **359**: 571–583.
- Williamson, E.B. 1899. Notes on Ohio Astacidae. Seventh Annual Report of the Ohio Academy of Science, pp 47–48. Ohio Academy of Science, Columbus, OH, USA.
- 14.110 Williamson, E.B. 1907. Notes on the crayfish of Wells County, Indiana, with the description of a new species. *Annual Report Department of Geology and Natural Resources of Indiana*, **31**: 749–763.
- 14.115
- 14.120
- 14.125
- 14.130
- 14.135
- 14.140
- 14.142